



Acute myeloid leukemia

FLT3 inhibitors in acute myeloid leukemia: ten frequently asked questions

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Abstract

The FMS-like tyrosine kinase 3 (FLT3) gene is mutated in approximately one third of patients with acute myeloid leukemia (AML), either by internal tandem duplications (FLT3-ITD), or by a point mutation mainly involving the tyrosine kinase domain (FLT3-TKD). Patients with FLT3-ITD have a high risk of relapse and low cure rates. Several FLT3 tyrosine kinase inhibitors have been developed in the last few years with variable kinase inhibitory properties, pharmacokinetics, and toxicity profiles. FLT3 inhibitors are divided into first generation multi-kinase inhibitors (such as sorafenib, lestaurtinib, midostaurin) and next generation inhibitors (such as quizartinib, crenolanib, gilteritinib) based on their potency and specificity of FLT3 inhibition. These diverse FLT3 inhibitors have been evaluated in myriad clinical trials as monotherapy or in combination with conventional chemotherapy or hypomethylating agents and in various settings, including front-line, relapsed or refractory disease, and maintenance therapy after consolidation chemotherapy or allogeneic stem cell transplantation. In this practical question-and-answer-based review, the main issues faced by the leukemia specialists on the use of FLT3 inhibitors in AML are addressed.

What is the role of the FLT3 pathway in the pathogenesis of acute myeloid leukemia?

The FMS-like tyrosine kinase 3 (FLT3) gene is located on chromosome 13q12. It belongs to the receptor tyrosine kinase (RTK) family that also comprises KIT, FMS, and PDGFR, among other receptors that play a major role in the regulation of hematopoiesis [1, 2]. The binding of FLT3 ligand to its extracellular domain activates downstream

signaling pathways such as MAPK and PI3K/protein kinase B-signals responsible for survival, maturation, and proliferation of hematopoietic cells [1, 3, 4]. The FLT3 receptor is overexpressed in most acute leukemias [5]. It is mutated in more than one-third of AML cases [2], representing one of the most prevalent molecular genetic alterations and has therefore proven to be an attractive therapeutic target. FLT3 mutations are involved in clonal evolution of AML and represent a second-hit driver through cooperation with other somatic intrinsic events [2, 6]. Two main types of FLT3 mutations are identified in newly diagnosed AML patients; internal tandem duplication (ITD) of the FLT3 juxta-membrane domain which are gain-of-function mutations found in 25–35% of AML patients [2], and tyrosine kinase domain (TKD) point mutations resulting in single amino-acid substitutions that mostly involve the aspartic acid 835 of the kinase domain, seen in 5–10% of patients [7]. ITD mutations interfere with the negative regulatory function of the juxta-membrane region, and kinase domain point mutations involve the activation loop, resulting in the loss of the auto-inhibitory function with subsequent constitutive activation of FLT3 kinase and its downstream proliferative signaling cascades involving JAK/STAT, MAPK, RAS, MEK, AKT/ERK, and PI3K, which inhibit apoptosis and promote further proliferation [8–12].

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What are the available FLT3 inhibitors and how do they work?

FLT3 inhibitors are tyrosine kinase inhibitors (TKI) classified into first and next generation inhibitors based on their potency and specificity for FLT3 and their associated downstream targets. First-generation inhibitors, including sunitinib, sorafenib, and midostaurin, are relatively non-specific for FLT3, as they possess extended activity involving other potential targets such as KIT, PDGFR, VEGFR, RAS/RAF, and JAK2 kinases. The off-target activities may contribute to a generally higher toxicity profile and clinical efficacy in non FLT3-mutated AML, but decreased efficacy in mutated FLT3 with high allelic burden [13]. The next generation inhibitors, including quizartinib, crenolanib, and gilteritinib, are more specific and potent, with a lower half-maximal inhibitory concentration (IC₅₀) and fewer toxicities associated with off-target effects. Furthermore, FLT3 inhibitors are categorized as Type I and Type II based on their mechanism of interaction with the receptor [14]. These agents interact with the ATP-binding site of the intracellular TKD and competitively inhibit this site of the enzyme. Type I inhibitors including sunitinib, midostaurin, lestaurtinib, crenolanib, and gilteritinib, bind to the ATP-binding site when the receptor is in the active conformation, while Type II inhibitors like sorafenib, ponatinib, and quizartinib, interact with a hydrophobic region directly adjacent to the ATP-binding domain that is only accessible when the receptor is inactive and they prevent receptor activation. As previously mentioned, the most common site for TKD mutations is D835 which occurs by single amino acid exchanges at the activation loop residues aspartate 835. TKD mutations favor the active conformation of the receptor and alter TKI binding. Consequently Type I inhibitors prevent activity in AML cells against both ITD and TKD with generally stronger binding to the FLT3-ITD-mutated kinase, while Type II inhibitors target ITD but lack efficiency against TKD mutations, though the development of TKD mutations, in particular D835 in AML cells with ITD have proved to be a mechanism of acquired, or secondary resistance to Type II FLT3 inhibitors [14–17]. These agents have been evaluated in many phase II–III clinical trials, some of them like midostaurin and gilteritinib, have US Federal Drug Administration (FDA) approval and others are currently not being developed for AML or no longer available (sunitinib and lestaurtinib) (Table 1).

What is the prognostic impact of FLT3-mutation?

The prognostic impact of FLT3-TKD mutations is still not well defined in patients with de novo AML [18–20].

In contrast, the unfavorable impact of FLT3-ITD on prognosis is well established; it is associated with a poor prognosis in terms of risk of relapse and overall survival (OS) [21]. Moreover, the prognostic impact of FLT3-ITD mutation may also be influenced by mutant-to-wild-type allelic ratio (AR), insertion site, ITD length, karyotype, as well as concomitant mutations such as NPM1, DNMT3A, WT1, and RUNX1 [22–25]. FLT3-ITD mutations with a higher AR have been associated with dismal outcomes in some studies but not in others [24, 26, 27]. For instance, Schlenk et al. [24] reported that high AR (≥ 0.51) and a FLT3-ITD insertion site in TKD1 in newly diagnosed FLT3-ITD-mutated AML, predicted low complete remission (CR) rates and poor OS. Similarly, in another study a threshold of AR of >0.78 was significantly associated with shorter OS and disease-free survival (DFS) [26]. Notably, FLT3 inhibitors were not used in both studies. In contrast, analysis of a large cohort of patients in Medical Research Council trials revealed no correlation between the risk of relapse and AR [27]. In addition, AML patients with a low (AR < 0.5) FLT3-ITD AR (FLT3-ITD^{low}) and NPM1 mutations have a favorable prognosis as designated by the European Leukemia Net and the National Comprehensive Cancer Network consensus panels [28, 29]. Schnittger et al. [30] reported a 3-year-OS rate of 60% in AML patients with low AR FLT3-ITD and NPM1 mutations. Conversely, other studies have shown that patients with both low and high AR FLT3-ITD mutated AML experienced similarly poor outcomes regardless of NPM1 status and all patients should be referred to allo-HCT [31, 32].

Which FLT3 inhibitors to use with induction chemotherapy in patients with newly diagnosed FLT3-mutated AML?

Several FLT3-TKIs have been evaluated in combination with intensive cytarabine and anthracycline-based induction in newly diagnosed AML patients (Table 2).

Sorafenib is a potent multi-kinase inhibitor that was evaluated in many clinical trials in combination with induction chemotherapy in AML patients. Ravandi et al. reported a high response rate in patients with previously untreated AML who received a combination of sorafenib, cytarabine, and idarubicin. In the group of patients with FLT3-ITD mutations, CR with incomplete recovery rates were 95%, and DFS and OS were 13.8 and 29 months, respectively [33, 34]. However, in another study, sorafenib combined with standard 7 + 3 chemotherapy did not improve EFS or OS among elderly patients (>60 years) with newly diagnosed AML [35]. Conversely, in a phase II, randomized controlled trial (RCT) in younger patients (≤ 60 years), frontline sorafenib combined with standard

Table 1 Summary of available FLT3 inhibitors.

| Kinase inhibitory profile | Dose | IC50 for FLT3-ITD | FLT3 TKD activity | Toxicity profile | Developmental phase/clinical indication |
|--|--------------|-------------------|-------------------|---|--|
| First-generation (FLT3 non-selective) | | | | | |
| Sumitinib c-KIT, VEGFR2, PDGFR β , RET | 50 mg daily | <10 nM | + | Fatigue, anorexia, diarrhea, nausea, mucositis, HTN | Phase II Significant toxicities and short remissions Not currently being developed for AML |
| Sorafenib RAF/MEK/ERK VEGFR1/2/3, BRAF PDGFR β , KIT, RET | 400 mg BID | 58 nM | - | Skin rash, fatigue, diarrhea, nausea, HTN | Phase II-III Off-label use Maintenance post allo-HSCT for FLT3-mutated AML Front-line setting in combination with HMA for unfit FLT3-mutated AML |
| Midostaurin PKC, SYK, FLK-1, AKT, PKA, c-KIT, FGR, SRC, PDGFR α/β , VEGFR1/2 | 50 mg BID | <10 nM | + | Fever, edema, infections, nausea, vomiting, diarrhea, fatigue, bleeding | Phase III Approved for front-line FLT3-mutated AML (ITD and TKD) in combination with chemotherapy FDA approved on April 27, 2017 |
| Next-generation (FLT3 selective) | | | | | |
| Crenolanib PDGFR α/β | 100 mg TID | 1.3 nM | + | Nausea, vomiting, transaminitis, rash infections, edema | Phase III Front-line FLT3-mutated AML (ITD and TKD) and RR-AML in combination with chemotherapy No FDA approval so far |
| Quizartinib SCFR/KIT, PDGFR CSF1R/FMS | 60 mg daily | 1.3 nM | - | QTc prolongation, myelosuppression, nausea, vomiting | Phase III RR FLT3-ITD AML as monotherapy Front-line FLT3-mutated AML with 7+3 No FDA approval but approved in Japan |
| Gilteritinib LTK, ALK, AXL | 120 mg daily | 0.29 nM | + | Diarrhea, fatigue, transaminitis, febrile neutropenia, infections | Phase III Approved for RR FLT3-mutated AML as monotherapy Front-line FLT3-mutated AML with AZA for unfit patients FDA approved on November 28, 2018 |

HTN hypertension, HMA hypomethylating agents, RR relapsed refractory

Table 2 Studies of *FLT3* inhibitors and chemotherapy for newly diagnosed *FLT3*-mutated AML patients.

| Ref. | Protocol | Study phase/ design | N | Age, yrs | FLT3 | Response/DFS/ EFS/RFS | Overall survival |
|-------------------------|---|-------------------------|--------------------------|------------|--------------------------------------|---|---|
| Ravandi et al. [33, 34] | 1–2 cycles <i>Ida</i> / <i>Ara-c</i> induction followed by max 5 cycles <i>Ida</i> / <i>Ara-c</i> cons + sorafenib | Phase II Non-randomized | 62 | 53 (18–66) | Wild type (63%) Mutated (37%) | CR/Cri: 95% (FLT3-ITD) DFS: 13.8 mo (all patients) DFS: 9.9 mo (FLT3-ITD) | 29 mo (all patients) 15.5 mo (FLT3-ITD) 3-year OS: 48% (all patients) |
| Serve et al. [35] | 7+3 induction followed by 2 cycles of intermediate/high-dose cytarabine cons + sorafenib or placebo | Phase III, RCT | 197 | >60 | Wild type FLT3-ITD (14%) | Median EFS: 7 mo for placebo vs 5 mo for sorafenib (NS) | Median OS: 15 mo for placebo vs 13 mo for sorafenib (NS) |
| Rollig et al. [36, 37] | 2 cycles of 7+3 induction followed by 3 cycles of HIDAC cons + sorafenib or placebo | Phase II, RCT | 267 | ≤60 | Wild type (83%) Mutated (17%) | Median EFS: 26 mo for sorafenib vs 9 mo for placebo ($p = 0.005$) Median RFS: 63 mo for sorafenib vs 22 mo for placebo ($p = 0.033$) | 5-year OS: 61% for sorafenib vs 52% for placebo (NS) |
| Knapper et al. [38] | 1–2 cycles of induction chemotherapy followed by 3–4 cycles cons + lestaurtinib or placebo | Phase III, RCT | 500 | <60 | Mutated | 5-year RFS: lestaurtinib 40% vs placebo 36% (NS) | 5-year OS: lestaurtinib 46% vs placebo 45% (NS) |
| Stone et al. [39] | 1–2 cycles of 7 + 3 induction followed by 4 cycles HIDAC cons + midostaurin or placebo | Phase III, RCT | 717 | <60 | FLT3-ITD (77.5%) FLT3-TKD (22.5%) | Median EFS: 8.2 mo for midostaurin vs 3 mo for placebo ($p = 0.002$) Median DFS: 26.7 mo for Midostaurin vs 15.5 mo for placebo ($p = 0.01$) | 4-year OS: midostaurin 51.4% vs placebo 44.2% ($p = 0.009$) |
| Wang et al. [41] | Crenolanib + 7 + 3 | Phase II | 29 | ≤60 | Mutated | CR: 83% 2 relapses after 14 mo follow-up | – |
| Altman et al. [43] | Dose-escalation: Quizartinib (30–60 mg/d) plus 7 + 3 induction | Phase I | 19 | ≤60 | FLT3-ITD (47%) Wild type (53%) | Any response: 84% CRc: 74% | – |
| Pratz et al. [44] | Gilteritinib + induction (<i>Ida</i> / <i>Ara-c</i>) | Phase I | 62 | 59 (23–77) | Mutated (53%) FLT3-ITD (37%) | CRc: 90% (All FLT3-mutated) CRc: 100% (FLT3- mutated and dose 120 mg/d) Median DFS: 297 d | NR |
| Schlenk et al. [40] | 7 + 3 induction followed by HIDAC cons and transplant + Midostaurin followed by maintenance Midostaurin | Phase II prospective | 284 (≤60,198; >60,86) | 54 (18–70) | FLT3-ITD + | CR/Cri: 76% | 2-year EFS: Younger: 39% Older: 34% 2-year OS: Younger: 53% Older: 46% |

Yrs years, *mo* months, *Ida* idarubicin, *Ara-c* cytarabine, *cons* consolidation, *RCT* randomized controlled trial, *NS* not significant, *NR* not reached, *CRc* composite complete remission, *CRi* complete remission with incomplete hematologic recovery, *EFS* event-free survival, *OS* overall survival, *DFS* disease-free survival

induction chemotherapy significantly prolonged EFS (26 months) and relapse-free survival (RFS) (63 months) as compared with placebo plus chemotherapy (9 and 22 months, respectively). Notably, only 17% of these patients had FLT3-mutated AML [36, 37]. Lestaurtinib is another multi-kinase inhibitor that failed to demonstrate any overall clinical benefit in a phase III trial when combined with intensive chemotherapy in patients with newly diagnosed FLT3-ITD-mutated AML [38].

Midostaurin is a first-generation multi-kinase inhibitor of FLT3-ITD and TKD. This agent is the first FLT3 inhibitor shown to improve survival in FLT3-mutated AML based on the RATIFY trial. Hence, it was approved by the FDA in April 2017 for the treatment of adult patients with newly diagnosed FLT3-mutated AML. The RATIFY trial [39] was a multicenter phase III pivotal trial of 717 patients (age 18–59 years) with newly diagnosed FLT3-mutated AML. Patients were randomized to either placebo or midostaurin dosed at 50 mg orally twice daily on days 8–21 of each cycle of induction (7 + 3) and consolidation with high dose cytarabine (HIDAC) chemotherapy. Patients who remained in remission after completion of consolidation therapy received continuous daily midostaurin maintenance therapy for up to 1 year and transplantation was not mandated in the protocol. This study showed a significant EFS (8.2 months for midostaurin vs 3 months for placebo) and 4-year OS (51.4% for midostaurin vs 44.2% for placebo). Moreover, there was a trend toward better OS in all FLT3-mutation subtypes (TKD, ITD low AR, and ITD high AR) in the midostaurin arm [39]. In another recent phase II trial by Schlenk et al. [40], midostaurin was added to intensive chemotherapy induction and consolidation and was continued as maintenance in 284 newly diagnosed FLT3-ITD AML patients comprising 198 younger patients (18–60 years), and 86 older patients (61–70 years). CR plus complete remission with incomplete hematologic recovery (CRi) after induction therapy was observed in 76.4% (younger, 75.8%; older, 77.9%). Among these, 72.4% proceeded to allo-HCT. Of the whole group, 97 patients (34%) received maintenance therapy. The 2-year EFS and OS were 39 and 34%; 53 and 46% in younger and older patients, respectively (Fig. 1).

Many early phase trials combining the more potent next generation FLT3-TKIs with 7 + 3 induction chemotherapy in the frontline setting have been reported recently with meaningfully high response rate.

Crenolanib, a potent type I FLT3 specific inhibitor, was investigated in a phase II trial in combination with 7 + 3 induction therapy in 29 young patients (<60 years) with newly diagnosed FLT3-mutated AML. Of note, crenolanib was well tolerated with 84% of patients tolerating full dose during induction. CR was achieved in 72% (21/29) after one cycle of induction. After a median follow-up of

14 months, 23 of the 24 patients in CR were still alive [41]. Moreover, Goldberg et al. [42] demonstrated that the addition of crenolanib to induction chemotherapy in patients with concurrent FLT3 and other mutations (NPM1 + DNMT3A, RUNX1, or WT1) can overcome the poor prognostic implication of adverse mutations co-occurring with mutated FLT3.

In a phase I dose-escalation trial, quizartinib was evaluated in combination with induction 7 + 3 chemotherapy in 19 AML patients unselected for FLT3 mutational status. Maximum tolerated dose (MTD) was identified as 40 mg × 14 days (lowest dose level). The authors reported an 84% response rate and 74% composite CR (CRc) [43]. In 2018, Pratz et al. reported the updated results of a phase I/II study of gilteritinib combined with 7 + 3 and HIDAC consolidation in 62 unselected AML patients. The MTD and the recommended expansion dose were established at 120 mg/day and were associated with a CRc rate of 100% in FLT3-mutated patients and a median DFS of 297 days [44].

In summary, midostaurin is the only approved FLT3 inhibitor in combination with induction chemotherapy in newly diagnosed FLT3-mutated AML, based on the RATIFY trial. Midostaurin can be used regardless of FLT3 mutation settings (ITD, TKD, ITD with NPM1 mutation, FLT3-ITD^{low}, ITD with poor prognostic driver mutations). However, based on the fact that a very high response rate was achieved with next generation FLT3 inhibitors (80–90%), two phase III randomized trials are ongoing and investigating frontline quizartinib + 7 + 3 chemotherapy (NCT02668653), and crenolanib vs midostaurin and 7 + 3 chemotherapy (NCT03258931). These studies could be practice-changing.

Is there a role for combination of FLT3 inhibitors and hypomethylating agents (HMA) in FLT3-mutated AML patients?

Almost 50% of AML patients are not candidates for intensive chemotherapy at diagnosis or at relapse due to poor performance status, medical comorbidities, advanced age or personal preference. Hence, FLT3 inhibitors are also being evaluated in combination with less intensive approaches in both the upfront and relapsed/refractory (RR) settings (Table 3). Ravandi et al. conducted a phase II study in 43 older patients with FLT3-ITD mutant relapse/refractory (RR)-AML treated with sorafenib 400 mg BID in combination with azacitidine (AZA) [45]. The overall response rate (ORR) was 46% (CR: 16%; CRi: 27%). Despite the encouraging response rate, the median duration of response was only 2.3 months and the median OS was 6.2 months. Strati et al. [46] evaluated in a phase 1/2 study, midostaurin plus AZA in 54 AML/high risk myelodysplasia

Fig. 1 Proposed treatment guidelines for FLT3 mutated AML patients.

| | Current Recommendation | Alternative Option | Ongoing Trials |
|-----------------------------|--|---|---|
| Front-line | Midaustorin + Standard Chemotherapy* (ITD and TKD) | Sorafenib + Standard Chemotherapy (ITD) | 1- Quizartinib + Chemotherapy 2- Crenolanib + Chemotherapy |
| Post-transplant Maintenance | Sorafenib (ITD) | Midaustorin (ITD and TKD) | - Gilteritinib - Crenolanib |
| Relapse / Refractory | Gilteritinib* (ITD and TKD) | - Quizartinib* - Sorafenib (ITD) | Crenolanib + Chemotherapy |

Table 3 Studies of FLT3 inhibitors and HMA for unfit FLT3 mutated AML patients.

| Ref. | Protocol | Study design | N | Age, yrs | Response | Survival |
|-------------------------|--|-----------------------------------|----|------------|--|--|
| Ravandi et al. [45] | Sorafenib + AZA RR | Phase 1/2 single-arm | 43 | 64 (24–87) | CR: 16% CRi: 27% | Median OS: 6.2 mo Median EFS: 3.8 mo |
| Strati et al. [46] | Midostaurin + AZA RR (76%) Unfit (24%) FLT3+ (74%) | Phase 1/2 single-arm | 54 | ≥ 65 (50%) | ORR: 26% CR: 2% Cri: 11% | Median OS: 5 mo |
| Swaminathan et al. [47] | Quizartinib + AZA or LDAC Newly diagnosed (age > 60) or RR (any age) | Phase 1/2 single-arm | 61 | 68 (23–84) | Q+AZA: CR: 22%, Cri: 41% Q+LDAC: CR: 8%, CRi: 29% | Median OS/ RFS: Q+AZA: 13.4 mo/6.9 mo Q+LDAC: 6.7 mo/3 mo |
| Esteve et al. [48] | Gilteritinib + AZA Newly diagnosed Unfit patients | Safety cohort of randomized trial | 15 | 76 (65–86) | ORR: 80% CR: 26.5% CRi: 40% | N/A |

RR relapsed refractory, CR complete remission, CRi complete remission with incomplete hematologic recovery, ORR overall response rate, OS overall survival, RFS relapse-free survival, AZA azacitidine, LDAC low dose cytarabine, Q Quizartinib, mo months, Yrs years

(MDS) patients either newly diagnosed and unfit ($n = 13$) or RR patients ($n = 41$). FLT3 mutation was positive in 74% of patients and half of them were ≥ 60 years. Midostaurin dose was 50 mg BID in the phase II cohort. After a median follow-up of 12 weeks, ORR was 26% (CR: 2%; CRi: 11%). Notably, the response rate was most prominent (33%) among the 27 patients with FLT3-ITD previously unexposed to FLT3 inhibitors. Median OS was 22 weeks and the regimen was generally well tolerated. In another phase I/II study [47], 61 patients with FLT3-ITD mutated AML, MDS, or chronic myelomonocytic leukemia were

treated with quizartinib (Q) + AZA or with Q + low dose cytarabine (LDAC). They included older patients (>60 y) for upfront treatment and patients at any age for first salvage treatment. Forty-nine patients were enrolled in the phase 2 part (Q + AZA: 31 patients, Q + LDAC: 18 patients). Quizartinib dose was 60 mg daily. The ORR was 73% (LDAC arm: 67%, AZA arm: 76%) and 92% (11 of 12) for previously untreated patients; the median survival was 18.6 and 11.25 months for previously untreated and treated patients, respectively. More recently Esteve et al. published a safety cohort of Gilteritinib plus AZA arm of the ongoing

clinical trial (NCT02752035) before randomization [48]. They assessed 15 older (≥ 65 years) unfit patients with FLT3-mutated AML treated with escalating doses of gilteritinib (80 or 120 mg/day) on Days 1–28 in combination with AZA. Grade ≥ 3 adverse events (AEs) occurred in $\geq 25\%$ of patients. Fatal AEs were observed in eight patients; however, none of these were related to treatment. The CRc rate was 67% ($n = 10$ of 15), CR was observed in four patients, and CRi in six patients.

In summary, there is lack of data demonstrating a benefit for the combination of HMA and FLT3 inhibitors in FLT3-mutated RR or unfit newly diagnosed AML patients, however some of the present studies showed that the combination appears safe and may improve both response rate and survival and should be further evaluated in larger studies.

Is there still a role for allo-SCT in FLT3-mutated AML patients in the era of FLT3 inhibitors?

Allo-HCT improves outcome of AML patients with FLT3-ITD when performed in CR1 [49–51]. Recent data confirmed that allo-HCT remains the best consolidation therapy for AML patients with FLT3-ITD, and hence should be offered as soon as possible in CR1 regardless of the use of FLT3 inhibitors, NPM1 status, FLT3 AR and donor types. Dezer et al. [52] evaluated the role of allo-HCT in 133 AML patients, 31 (23%) harbored FLT3-ITD mutation. There was significantly better RFS in FLT3-ITD mutated patients treated with transplant as compared with non-transplant group (54 months vs 8.6 months). In another study by Oran et al., post-remission treatment with consolidation chemotherapy and allo-HCT were compared in 227 FLT3-mutated AML patients who achieved CR1 after induction chemotherapy. Transplant improved both RFS and OS regardless of NPM1 and FLT3 AR at diagnosis (≥ 0.3 vs < 0.3) [53]. Furthermore, MDACC group analyzed the outcome of allo-HCT in 200 FLT3-mutated AML patients (FLT3-ITD: 83%, FLT3-TKD: 17%; CR1: 49%, CR2: 10%) and showed a dramatic increase in the relapse rate and worse PFS for patients beyond CR1 [54]. Non-relapse mortality was higher in patients in CR2 than in CR1 (HR = 3, $P = 0.02$). In the same study, HLA-matched donor transplants had similar survival with haploidentical transplants and no difference in outcomes was observed between FLT3 ITD or FLT3 TKD mutations on multivariate analysis. Albeit addition of FLT3 inhibitors improves the outcome of FLT3 mutated AML, allo-HCT specially in CR1 still has a significant role. In the Ratify trial and although allo-HCT was not mandated in the study protocol, more than half of patients received transplant at some point

during the disease course. Indeed, allo-HCT was performed after CR1 in 28.1% of the patients in the midostaurin group and in 22.7% in the placebo group. Furthermore, a sensitivity analysis of the primary end point was performed after censoring patients at the time of transplant to reduce the influence of allo-HCT on OS and result shows a trend toward better survival ($p = 0.07$) in the midostaurin patient group who received an allo-HCT in CR1, and a significant decrease in cumulative incidence of relapse ($p = 0.02$) in all patients achieving a CR after induction therapy [39].

When to use FLT3 inhibitors as maintenance therapy including after allo-HCT?

As stated before, although allo-HCT improves outcome of AML patients with FLT3-ITD when performed in CR1, many patients still relapse and even have higher rates of early relapse post-transplant [49, 50]. The use of FLT3 inhibitors in the maintenance setting after allo-HCT is supported by the observation of an antileukemic synergism between sorafenib and allo-reactive donor cells (Table 4) [55]. Sorafenib was the first TKI investigated in the setting of post-transplant maintenance therapy in AML patients with FLT3-ITD mutation [50, 55–60].

Chen and colleagues [57] reported the results of the first phase 1 trial of sorafenib after allo-HCT in 22 patients with FLT3-ITD AML. The investigators used a dose escalation design to define the MTD. They found that sorafenib can be safely used after transplantation with a MTD of 400 mg twice daily. The most common observed adverse events were skin rash and gastrointestinal toxicities. Acute skin graft-versus-host disease (GVHD) grade II was observed in one case after starting sorafenib and the incidence of chronic GVHD was 38%. The study demonstrated a 1-year DFS and OS of 85% and 95% respectively, after allo-HCT. Antar et al. [58] reported the first pilot report on six patients who received sorafenib mostly as maintenance after transplantation. Grade II skin GVHD was observed in five of six patients. All six patients were alive and in CR after a median follow-up of 16 months (range, 10–29 months) and all patients were in molecular remission. In a single institution study, Brunner et al. [59] retrospectively evaluated the efficacy of sorafenib maintenance in patients with FLT3-ITD AML at diagnosis, who underwent allo-HCT in CR1. Patients on sorafenib maintenance ($n = 26$) had an improved 2-year OS compared with historical controls ($n = 54$) (83% vs 58%, $p = 0.019$). In a multi-institution study by Battipaglia et al., sorafenib was used as posttransplant maintenance in 28 adults with FLT3 positive AML [60]. Twenty-five patients received sorafenib as primary prophylaxis. DFS and OS at one year were 91% and 89%, respectively. A recent update of this study showed a 2-year

Table 4 Studies of *FLT3* inhibitors as maintenance in *FLT3* mutated AML patients.

| Ref. | FLT3 Inh | Study design | Patients, <i>N</i> | Age, Yrs | Response |
|--------------------------------------|--------------------|------------------------------|--|--|--|
| Chen et al. [57] | Sorafenib | Phase I | 22 | 54 (20–67) | 2-yr OS: 78% 2-yr PFS: 72% |
| Antar et al. [58] | Sorafenib | Retrospective | 6 | 50 (32–58) | 6 (100%) of pts are alive with a median follow-up of 16 mo |
| Brunner et al. [59] | Sorafenib | Retrospective 2 arms | 80 (Sorafenib: 26; Control: 54) | Sorafenib: 54.5 (20–74) Control: 53 (25–72) | 2-yr OS: 83% for Sorafenib, 58% for control (S) 2-yr DFS: 85% for sorafenib, 52% for control (S) |
| Battipaglia et al. [60, 61] | Sorafenib | Retrospective Multi-center | 28 (Primary prophylaxis: 25, Secondary prophylaxis: 3) | 45 (16–57) | 1-yr OS: 89±7% 1-yr LFS: 91±6% 2-yr OS: 80 ±8% 2-yr PFS: 73±9% |
| Bazarbachi et al. [62] | Sorafenib | EBMT registry-based analysis | 462 (prophylaxis:19; preemptive:9; Control 434) | 50 (18–78) | Matched-pair analysis 26 sorafenib pts and 26 controls: 2-yr LFS: 79% (sorafenib) and 54% (control) (S) 2-yr OS: 83% (sorafenib) and 62% (control) (S) |
| Burchert et al. (SORMAIN trial) [63] | Sorafenib | Phase II Prospective RCT | 83 Sorafenib: 43 Placebo: 40 | 54 (18–75) | 2-yr RFS: 85% (sorafenib) and 62% (Placebo) (S) |
| Maziarz et al. (Radius trial) [65] | Midostaurin | Phase II randomized | 60 Midostaurin + SOC: 30 Placebo + SOC: 30 | 18–70 | 1.5-yr RFS: 89% (Midostaurin + SOC) 1.5-yr RFS: 76% (Placebo + SOC) |

S significant, *N* number, *Yrs* years, *mo* months, *pts* patients, *RCT* randomized controlled trial, *SOC* standard of care, *OS* overall survival, *LFS* leukemia-free survival, *RFS* relapse-free survival

PFS and OS of 73% and 80%, respectively [61]. More recently Bazarbachi et al. [62] reported the results of the European Group for Blood and Marrow Transplantation (EBMT) registry-based study on 462 patients with FLT3-mutated AML (FLT3-ITD-95%). Among these patients, 62 received posttransplant sorafenib either as prophylactic ($n = 19$), preemptive therapy ($n = 9$), or as treatment for relapse ($n = 34$). On multivariate analysis, maintenance sorafenib significantly improved leukemia-free survival (LFS) (hazard ratio [HR] = 0.35), OS (HR = 0.36) and graft-versus-host disease-free, relapse-free survival (HR = 0.44). A matched-pair analysis was then performed on data from 26 patients in the sorafenib maintenance group and from 26 controls. After a median follow-up of 39 months, the 2-year LFS and OS were 79% and 83% in the sorafenib group, vs 54% and 62% for controls ($p = 0.002$ and 0.007 , respectively). Finally, in the SORMAIN study [63], a prospective double-blind RCT, 83 adult patients (aged 18–75 years) with FLT3-ITD AML who had undergone allo-HCT and were in confirmed CR after transplant, were randomized to either receive maintenance sorafenib ($n = 43$) at a dose 200–400 mg BID or placebo ($n = 40$) for up to 24 months. After a median follow-up of almost 42 months, the 2-year RFS was 85% in the sorafenib group compared with 53.3% in the placebo group ($p = 0.013$) and after a median follow-up of 55.4 months, OS was significantly longer in the sorafenib arm compared to the placebo arm ($p = 0.03$).

Midostaurin was also evaluated in the maintenance setting. In the RATIFY trial [39], only patients who were in remission after consolidation chemotherapy and who did not undergo allo-HCT were allowed to receive either midostaurin ($n = 360$) or placebo ($n = 357$) as maintenance therapy for up to 12 months. A post-hoc analysis of the RATIFY trial on maintenance midostaurin showed no significant difference in 1-year DFS and OS between the two arms and hence no conclusions could be made about the potential benefit of midostaurin maintenance therapy [64]. In contrast to the RATIFY trial, in a phase II prospective study by Schlenk and colleagues of 284 newly diagnosed FLT3-ITD AML patients, midostaurin maintenance therapy was administered after allo-HCT (56%) and after consolidation therapy (55%). In a landmark analysis of patients who underwent allo-HCT in CR1/Cri1 ($n = 134$) and who were event-free at day 100 post-transplant ($n = 116$), those starting maintenance therapy within 100 days after transplant ($n = 72$) had a significantly better EFS and OS ($p = 0.004$ and $p = 0.01$, respectively) compared to those who did not [41]. In another prospective randomized phase II trial [65], 60 FLT3-ITD mutated AML patients who underwent allo-HCT in CR1 were randomized to receive standard of care (SOC) with or without midostaurin ($n = 30$ each group) at 50 mg BID continuously (4 week cycles for

up to 12 cycles) starting 28–60 days post allo-HCT. RFS at 18 months post-allo-HCT in the midostaurin plus SOC and SOC alone arm were 89% and 76% respectively ($P = 0.26$).

Quizartinib was also evaluated in a phase I safety study [66]. Thirteen adult patients with FLT3-ITD-mutated AML in CR following allo-HCT received one of two quizartinib dose levels (40 mg/d $n = 7$, 60 mg/d $n = 6$), administered orally in 28-day cycles for up to 24 cycles. Almost 77% of patients had received quizartinib for more than one year and preliminary data indicated a lower rate of relapse compared with historical cohorts with only one relapse among the 13 patients.

Overall, FLT3 inhibitors, particularly sorafenib, are safe and significantly improve outcomes when used as maintenance therapy after allo-HCT. Sorafenib can be considered as SOC in that setting. Other clinical trials testing FLT3 inhibitors in the maintenance setting, particularly gilteritinib, are currently accruing (NCT02997202, NCT03379727, NCT02927262, NCT02668653, NCT02400255).

Which FLT3 inhibitors can be used in the relapsed/refractory setting including relapse after allo-HCT?

Most FLT3 inhibitors have been evaluated in RR-AML patients with *FLT3-ITD*. However, only second generation FLT3 inhibitors including quizartinib and gilteritinib that exhibit a high potency and specificity for the FLT3 kinases have achieved clinically meaningful responses in this category of patients. On the other hand, sorafenib has shown a unique effectiveness in the setting of relapse after allo-HCT (Table 5).

Quizartinib was evaluated in two phase II and one phase III trials [67–70]. In one phase II single arm study [67], patients with RR-AML were divided into two cohorts: aged ≥ 60 years with relapsed or refractory AML within 1 year after first-line therapy (cohort 1, $n = 157$) and those ≥ 18 years of age following prior salvage chemotherapy or allo-HCT (cohort 2, $n = 176$). Patients received quizartinib at a dose of 135 mg/day and 90 mg/day for men and women respectively. A significant QT interval corrected using Fridericia's formula (QTcF) prolongation was observed in 17% and 15% of patients treated with the 90 and 135 mg doses, respectively. In *FLT3 ITD* positive patients, 56% and 46% in cohorts 1 and 2 respectively, achieved CRc. Quizartinib was generally well-tolerated. Another phase IIb study randomized 76 RR-AML patients with FLT3 mutation, who had previously received allo-HCT or 1 second-line salvage therapy, into two doses of quizartinib (30 or 60 mg/day) [68]. CRc rates were 47% in both groups, comparable with the previous study with higher quizartinib doses. In contrast to this previous higher dose study, significant QTcF

Table 5 Studies of *FLT3* inhibitors in FLT3 mutant RR-AML.

| Ref. | FLT3 inhibitor | Study phase and design | Patients characteristics | Response | Survival |
|------------------------------------|---------------------|--|---|---|--|
| Metzelder et al. [55, 74] | Sorafenib | Retrospective | <i>N</i> : 65 Pre Allo-HCT: 36 Post Allo-HCT: 29 | Post Allo-HCT CR: 48% CRi: 21% CRc: 17% after 7.5 y follow-up | NA |
| Cortes et al. [67] | Quizartinib | Phase II single arm multicenter study | <i>N</i> : 333 Cohort 1 (157 pts): age ≥60 yrs within 1 yr after CR1. Cohort 2 (176 pts): ≥18 yrs after salvage chemo or allo-HCT | FLT3-ITD + pts: cohort 1: CRc: 56% CR: 3% cohort 2: CRc: 46% CR: 4% | NA |
| Cortes et al. [68] | Quizartinib | Phase IIb Randomized two dosing regimens | <i>N</i> : 76 Q1: 30 mg/d (50%) Q2: 60 mg/d (50%) after allo-HCT or one 2nd line salvage therapy age ≥ 18 yrs | CRc: 47% (both groups) | Median OS: Q1: 21w Q2: 27w |
| Cortes et al. (QuANTUM-R) [69, 70] | Quizartinib | Phase III, RCT 2:1 Q Vs SOC chemo | <i>N</i> : 367 Age ≥18 yrs FLT3 + CR1 ≤ 6 mo | CRc: -Q: 48% -SOC: 27% (S) | Median OS: Q: 6.2 mo SOC: 4.7 mo (S) |
| Perl et al. [71] | Gilteritinib | Phase I–II Dose escalation and expansion | <i>N</i> : 252 FLT3-ITD/ FLT3-D835 | ORR: 40% CR: 8% Cri: 18% | Median OS: 20 w ^a |
| Perl et al. (ADMIRAL trial) [72] | Gilteritinib | Phase III, RCT 2:1 Vs SOC chemo/AZA | <i>N</i> : 371 Age 62 yrs (19–85) FLT3-ITD: 88.4% FLT3-TKD: 8.4% | CR: G: 21% SOC: 10.5% | Median OS: G: 9.3 mo SOC: 5.6 mo 1-year-survival: G: 37% SOC: 16.7% |
| Bazarbachi et al. [62] | Sorafenib | EBMT registry-based analysis. | <i>N</i> : 152 (sorafenib 34; control 118) Age ≥18 yrs FLT3 + -relapse or progression after 1 allo-HCT | CR: S: 39% control: 33% (after 1st line) | Pair matched analysis (30 sorafenib and 30 controls) 2-yr-OS: Sorafenib: 38% control: 9% (S) |

N number, *yrs* years, *chemo* chemotherapy, *pts* patients, *CRc* composite complete response, *ORR* overall response rate, *NA* not available, *RCT* randomized controlled trial, *Q* Quizartinib, *w* week, *SOC* standard of care; *mo* month, *S* significant, *G* Gilteritinib, *S* sorafenib

^aIn patients who had received any prior TKI therapy

prolongation was only reported in 5% and 3% of patients treated with 30 and 60 mg/day, respectively. Median OS was 21 and 27 weeks for patients treated with 30 and 60 mg/day, respectively.

Based on these two studies, a phase III study (QUANTUM-R) [69, 70] recently reported the efficacy and safety of quizartinib as compared with other standard therapeutic options in FLT3-mutated RR-AML. In this multicenter trial, 367 adult *FLT3-ITD* mutated AML patients were randomized in a 2:1 ratio to receive either oral quizartinib (*n* = 245; 60 mg/day, with a 30 mg lead-in of 15 days) or salvage chemotherapy (*n* = 122; MEC, FLAG-Ida, LDAC). The median OS was 6.2 months with quizartinib compared with 4.7 months for salvage chemotherapy (HR = 0.67; *p* = 0.01). One-year OS was 27% in the quizartinib group

and 20% in the control group. Notably, these responses were overall including allo-HCT and 32% of patients who received quizartinib proceeded to allo-HCT vs only 12% of patients who received salvage chemotherapy.

Gilteritinib is another potent and highly selective FLT3 inhibitor with activity against both FLT3 ITD and FLT3-D835 TKD mutant receptors. In a Phase I–II trial [71], 252 adult RR-AML patients including 58 with wild-type FLT3 and 194 with FLT3 mutations (FLT3-ITD, *n* = 162; FLT3-D835, *n* = 16; FLT3-ITD and D835, *n* = 13; other, *n* = 3) received once daily oral gilteritinib at one of seven dose-escalation cohorts (20, 40, 80, 120, 200, 300, or 450 mg). Antileukemic activity was observed across all dose levels regardless of FLT3 mutation status (ORR = 40%) with significantly higher response rate (ORR = 52%)

among *FLT3*-ITD patients at gilteritinib doses ≥ 80 mg/day. More recently Perl et al. [72] reported the results of a randomized (2:1), open-label phase III trial (ADMIRAL) comparing gilteritinib monotherapy (120 mg once daily) vs SOC (LDAC, azacitidine, MEC, FLAG-IDA) in 371 *FLT3*-mutated RR-AML patients. Most of the patients were *FLT3*-ITD positive (88%). OS was significantly higher in the gilteritinib group than in the control group (9.3 months vs 5.6 months; $p = 0.0007$) and 1-year survival rates were 37.1% and 16.7%, respectively. The CR/CRi for gilteritinib and SOC groups were 34.0% and 15.3%, respectively ($p = 0.0001$).

Sorafenib was assessed retrospectively in few trials in patients with *FLT3*-ITD mutated AML who relapse post allo-HCT [55, 62, 73, 74]. Metzelder et al. [55, 74] described a more profound and sustained remission in a study of 29 *FLT3*-ITD AML patients, when sorafenib was given after allo-HCT (CR 48% and CRi 21%) with five patients (17%) achieving sustained CR after 7.5 years median follow-up. This finding may be explained by the antileukemic synergy between sorafenib and alloreactive donor cells. In the EBMT registry-based study, Bazarbachi et al. reported 152 *FLT3*-mutated AML adult patients who underwent allo-HCT at EBMT participating centers and who progressed or relapsed after a first allo-HCT. Among these, 34 patients received sorafenib as part of salvage therapy. On multivariate analysis, sorafenib given as salvage for relapse (time dependent) significantly improved OS (HR = 0.44; $p = 0.001$). Older age (HR = 1.2; $p = 0.04$), active disease at transplant (HR = 2.4; $p = 0.001$), and reduced intensity conditioning (HR = 1.76; $p = 0.01$) adversely affected OS. Time from transplant to relapse had no significant impact on OS (HR = 0.98; $p = 0.17$). A preplanned matched-pair analysis was then performed on data from 30 patients in the sorafenib group and from 30 controls. One and 2-year OS was 51% and 38% for patients in the sorafenib group vs 17% and 9% for controls, respectively (HR = 0.28; $p = 0.0001$).

In summary, gilteritinib is approved as single agent salvage therapy for patients with RR-AML *FLT3*-mutated AML. Quizartinib is not approved in US but in Japan in this setting. Sorafenib is not approved but can be used off-label as part of salvage therapy in these patients who relapse or progress after allo-HCT.

What are the main mechanisms of resistance in the *FLT3* pathway and how to overcome them?

Although *FLT3*-TKI therapy has clearly improved the historically poor outcomes of *FLT3*-mutated AML, clinical response to a *FLT3* inhibitor is short-lived in the RR setting

with ultimate progression in most patients. Generally, resistance to *FLT3*-TKIs may be classified as either inherent (primary) resistance of the malignant clone, or development of secondary (acquired) resistance emerging after an initial response [75]. This resistance can also be categorized into intrinsic and present before therapy, or extrinsic that emerges during or after therapy. Primary resistance is mainly due to insensitive *FLT3* mutations to specific TKIs or activation of alternative survival pathways (*FLT3* independent). Conversely, secondary resistance has several causes including resistant mutations in the ATP binding pocket, autocrine *FLT3* signaling, *FLT3* overexpression and activation of alternative pathways such as mutations of the *NRAS* gene. One cardinal and best described mechanism of resistance is the development of point mutations in the *FLT3* drug binding site, most commonly at residue *D835* of *FLT3-TKD* [15, 76]. There is still lack of data to recommend a screening of resistance-mediating mutations in case of relapse following induction, consolidation chemotherapy and maintenance with *FLT3* inhibitors. However, to overcome the emerging resistance to *FLT3*-TKIs, many combinatorial strategies involving *FLT3*-TKI to counteract resistant subclones are being evaluated and aim to induce deeper remissions and eradicate corresponding leukemia stem cells. One of the common combination strategies is *FLT3*-TKIs with epigenetic therapy including histone deacetylase inhibitors and HMAs, which demonstrated encouraging and synergistic antileukemic in vitro efficacy particularly by downregulation of the JAK/STAT pathway [77]. Another potential strategy to overcome resistance is by targeting the downstream pathways of *FLT3*-ITD by concomitant use of *FLT3* inhibitors and STAT5 inhibitor, Pim kinase inhibitors, CDK4/6 inhibitor, mTOR or Akt inhibitors [78–82]. Another strategy is the aspect of inhibition of *FLT3*-ITD glycosylation to modify *FLT3*-ITD downstream signaling with statins [83]. This represents a potential mechanism for circumventing resistance by targeting explicit downstream pathways [84]. One more strategy that has been suggested to overcome resistance, give sustained disease control and even potential cure after allo-HCT is the synergistic effect of *FLT3* inhibitors, particularly sorafenib, with alloreactive donor T-cells. This synergy with graft-versus-leukemia effect has been observed without an increase in GVHD [55, 62, 85].

What are the common adverse events related to *FLT3* inhibitors and how to manage them?

First-generation *FLT3* inhibitors including midostaurin and sorafenib are relatively nonspecific and generally have off-target activities. However, data from RATIFY trial [82]

showed no unexpected adverse events related to midostaurin. Grade 3 or 4 anemia, rash, and nausea were significantly higher in midostaurin group than in the placebo group (92.7% vs 87.8%, 14.1% vs 7.6% and 9.6% vs 5.6% respectively). Importantly, there was no dose modification for hematologic toxicity due to midostaurin during induction and consolidation therapy. Nevertheless, during maintenance therapy midostaurin was held if ANC is $<1000/\mu\text{l}$. Midostaurin was also held if QTc interval >500 ms or in case of grade ≥ 3 pulmonary toxicity. In the post transplant maintenance setting, data from the SORMAIN trial [63] revealed a generally manageable toxicity only by dose reduction, most common grade 3–4 adverse events in the sorafenib and placebo arms were acute GvHD (24% vs 18.2%), gastrointestinal toxicity (14.3% vs 15.4%), and infections (26.3% vs 23.1%). On the other hand, in the induction setting, data from the SORAML trial [36, 37] side effects that were significantly more common in the sorafenib group were fever, diarrhea, bleeding, cardiac events, hand–foot–skin reaction, and rash that led to withdrawal from study treatment in 30% of cases. The next generation inhibitors, including quizartinib, crenolanib and gilteritinib, are more specific and potent with generally manageable toxicities. Common treatment related adverse events with higher dose of quizartinib (≥ 90 mg/day) were mainly myelosuppression which is related to “off-target” activities (KIT inhibition) in contrast to gilteritinib and grade ≥ 3 QTcF prolongation (15%), which was reversible and successfully managed by dose reductions or treatment discontinuations. Lower doses of quizartinib were associated with significantly lower rates of grade ≥ 3 QTcF prolongation ($<5\%$) while maintaining the same efficacy [67]. Treatment related toxicities occurring in patients treated with crenolanib were grade 3 gastrointestinal toxicities, nausea, vomiting, transaminitis, and fluid retention [41]. Gilteritinib was associated with diarrhea (37%), anemia (34%), fatigue (33%), increased aspartate aminotransferase (26%), and increased alanine aminotransferase (19%) [72].

Conclusion

Midostaurin plus cytarabine and anthracycline induction chemotherapy and HIDAC consolidation chemotherapy is approved and should be offered to all newly diagnosed FLT3-mutated AML fit patients. Allo-HCT is recommended in CR1 for these patients and maintenance therapy after allo-HCT using off-label sorafenib should be strongly considered for 24 months. The combination of HMAs and FLT3 inhibitors is feasible in FLT3-mutated, unfit newly diagnosed or RR AML patients, but no strong data to support the benefit. Patients with RR FLT3-mutated AML can be salvaged by

gilteritinib or can be treated with sorafenib as part of salvage therapy particularly in the post transplant setting.

Compliance with ethical standards

Conflict of interest We declare the following conflicts of interest: Elias Jabbour (Research grants and advisory roles from Abbvie, Adaptive Biotechnologies, Amgen, BMS, Pfizer and Takeda), Mohamad Mohty (Honoraria from Novartis and Daiichi Sankyo), Ali Bazarbachi (Research grants and advisory roles from Novartis and Takeda). The other authors declare no conflict of interest. No financial support was provided for this project.

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